

**Claims**

1. A cytochrome c-reporter fusion protein construct comprising a modified cytochrome c protein or any functional analogue thereof derived from wild type cytochrome c, wherein  
5 said modified cytochrome c targets the mitochondria and has a reduced ability to induce apoptosis in a living cell.
2. The fusion construct of claim 1, wherein the modified cytochrome c binds apoptosis protease activation factor-1 (Apaf-1) at least ten times less than wild type cytochrome c.
- 10 3. The fusion construct of claim 1 or 2, wherein the modified cytochrome c binds apoptosis protease activation factor-1 (Apaf-1) at least 100 times less than wild type cytochrome c.
- 15 4. The fusion construct of any preceding claim, wherein the modified cytochrome c binds apoptosis protease activation factor-1 (Apaf-1) at least 1000 times less than wild type cytochrome c.
- 20 5. The fusion construct of any preceding claim, wherein at least one of the amino acids of the modified cytochrome c at positions 4, 7, 8, 25, 39, 62, 63, 64, 65 and 72 has been mutated relative to the wild type cytochrome c.
- 25 6. The fusion construct of claim 5, wherein the modified cytochrome c has an amino substitution or substitutions selected from the group consisting of K4E, K72A, K72L, K72R, K72G, K72X, E62N, K7E-K8E, K25P-K39H, K7A-E62N-K25P, K7A-E62N-K39H, K7E-K8E-E62N, K7A-K25P-E62N, K7A-E62N-K25P-K39H, E62N-T63N-L64M-M65S, K7E-K8E-E62N-K25P-K39H, K7E-K8E-K25P-E62N-T63N-L64M-M65S, K7E-K8E-K39H-E62N-T63N-L64M-M65S and K7E-K8E-K25P-K39H-E62N-T63N-L64M-M65S.
- 30 7. The fusion construct of claim 6, wherein the modified cytochrome c comprises the amino acid substitution selected from the group consisting of K7E-K8E-E62N-K25P-K39H, K7E-K8E-K25P-E62N-T63N-L64M-M65S, K7E-K8E-K39H-E62N-T63N-L64M-M65S and K7E-K8E-K25P-K39H-E62N-T63N-L64M-M65S.

8. The fusion construct of claim 6, wherein the modified cytochrome c comprises the amino acid substitution selected from the group consisting of K72A, K72L, K72R, K72G and K72X, wherein X represents trimethylation.

9. The fusion construct of either of claims 6 or 8, wherein the modified cytochrome c comprises the amino acid substitution K72A or K72L.

10. The fusion construct of claim 6, wherein the modified cytochrome c comprises the amino acid substitution K4E.

11. The fusion construct of any preceding claim, wherein the reporter is a fluorescent protein or a functional analogue thereof.

12. The fusion construct of claim 11, wherein said fluorescent protein is selected from the group consisting of Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP), Blue Fluorescent Protein (BFP), Cyan Fluorescent Protein (CFP), Red Fluorescent Protein (RFP), Enhanced Green Fluorescent Protein (EGFP) and Emerald.

13. The fusion construct of either of claims 11 or 12, wherein the fluorescent protein is Enhanced Green Fluorescent Protein or Emerald.

14. The fusion construct of claim 11, wherein the GFP comprises

- i) an amino acid substitution at position F64L;
- ii) an amino acid substitution at position S175G; and
- iii) an amino acid substitution at position E222G.

15. The fusion construct of any preceding claim selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 6.

16. The fusion construct according to any of claims 1 to 10, wherein the reporter is localisable by a detectable luminescent, fluorescent or radio-active moiety.

17. The fusion construct according to claim 16, wherein the reporter comprises an immunogenic motif.

5 18. The fusion construct according to claim 15 or 16, wherein the reporter comprises a cysteine-rich motif.

19. The fusion construct according to claim 16, wherein said detectable moiety comprises a bi- arsenical compound.

10 20. The fusion construct according to claim 16, wherein said moiety comprises an antibody.

21. A nucleotide sequence encoding a fusion construct of any of the preceding claims.

15 22. A nucleotide sequence encoding a fusion construct according to claim 15 selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO 5.

20 23. A nucleic acid construct comprising a suitable control region and a nucleotide sequence according to claim 21 or 22, said sequence being under the control of said control region.

25 24. A nucleic acid construct according to claim 23 being under the control of a promoter selected from the group consisting of native cytochrome c promoter, mammalian constitutive promoter, mammalian regulatory promoter, human ubiquitin C promoter, viral promoter, SV40 promoter, CMV promoter, yeast promoter, filamentous fungal promoter and bacterial promoter.

30 25. A nucleic acid construct according to claim 24, wherein said viral promoter is the CMV or the SV40 promoter.

26. A nucleic acid construct according to claim 24, wherein the promoter is the human ubiquitin C promoter.

27. A replicable vector comprising a nucleic acid construct according to any of claims 23 to 26.

28. The replicable vector of claim 27, wherein said vector is a plasmid vector.

29. The replicable vector of claim 27, wherein the vector is a viral vector.

30. The replicable vector of claim 29, wherein said viral vector is selected from the group consisting of cytomegalovirus, Herpes simplex virus, Epstein-Barr virus, Simian virus 40, Bovine papillomavirus, Adeno-associated virus, Adenovirus, Vaccinia virus and Baculovirus vector.

31. A host cell stably transformed with a nucleic acid construct according to any of claims 23 to 26.

32. A host cell transiently transformed with a nucleic acid construct according to any of claims 23 to 26.

33. The host cell of claims 31 or 32 selected from the group consisting of plant, insect, nematode, bird, fish and mammalian cell.

34. The host cell of claim 33, wherein said mammalian cell is a human cell.

35. The host cell of claim 34, wherein said human cell is selected from the group consisting of Hek, HeLa, U2OS and MCF-7.

36. The host cell of claim 35, wherein said Hek cell is Hek293.

37. The host cell according to any of claims 31 to 36 capable of expressing the fusion protein of any of claims 1 to 20.

38. A method for detecting apoptosis in a living cell comprising the steps of

i) culturing a cell transformed to over-express a fusion construct according to any of claims 1 to 20;

ii) determining the localisation of the fusion construct within the cell with time;

5 wherein a change in localisation of the fusion construct within the cell is indicative of apoptosis.

39. A method for measuring the effect that an agent has upon modulating apoptosis in a living cell comprising the steps of

10 i) culturing a cell transformed to over-express a fusion construct according to any of claims 1 to 20;

ii) determining the localisation of said construct within the cell;

iii) treating the cell with said agent and determining the localisation of the construct within the cell;

15 wherein any difference in the localisation of the construct within the cell relative to control cells untreated with the agent is indicative of the effect that the agent has upon modulating apoptosis.

40. A method for measuring the effect an agent has upon modulating apoptosis in a living cell comprising the steps of

20 i) culturing a first cell and a second cell which both over-express a fusion construct according to any of claims 1 to 20;

ii) treating said first cell with said agent and determining the localisation of said construct within the first cell;

25 iii) determining the localisation of the construct within said second cell which has not been treated with the agent;

wherein any difference in the localisation of the construct within the first cell and second cell is indicative of the effect that the agent has upon modulating apoptosis.

30 41. A method for measuring the effect an agent has upon modulating apoptosis in a living cell comprising the steps of

i) culturing a cell transformed to over-express a fusion construct according to any of claims 1 to 20;

ii) treating said cell with said agent and determining the localisation of the construct within the cell;

iii) comparing the localisation of the construct in the presence of the agent with a known value for the localisation of the construct in the absence of the agent;

5 wherein any difference in the localisation of the construct within the cell in the presence of the agent and said known value in the absence of the agent is indicative of the effect that the agent has upon modulating apoptosis.

42. The method according to claim 41, wherein the known value is stored on a database.

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43. The method according to any of claims 38 to 42, wherein the localisation of said fusion construct is measured by its luminescence, fluorescence or radioactive properties.

44. The method according to any of claims 39 to 43, wherein said agent induces  
15 apoptosis.

45. The method according to any of claims 39 to 43, wherein the agent inhibits  
apoptosis.

20 46. The method according to any of claims 38 to 45, wherein the localisation of the protein fusion is determined following fixation of the cells.

47. The method according to any of claims 38 to 46, where the agent is a chemical,  
physical or biological agent.

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